Orthopaedic Trauma Research Program (OTRP)
2006 Funded Proposals

1. Mechanisms of Heterotopic Ossification and Characterization of Preventive Therapeutics

2. Polymicrobial Chronic Infection Including Acinetobacter baumannii in a Plated Segmental Defect in the Rat Femur

3. Novel Therapies for Acinetobacter Osteomyelitis

4. Development of Military Field/Hospital Therapeutic Anti-Infective, Antibiofilm Acute Wound Care Gel Product

5. Prevention and Treatment of Heterotopic Ossification

6. Improved Healing of Infected Segmental Bone Defects through Controlled Delivery of FGF-2, PDGF, and Tobramycin

7. Adipose-derived Mesenchymal Stem Cells for Treatment of Large Bone Defects

8. Cellular Therapy to Obtain Rapid Endochondral Bone Formation

9. New Bone Formation in a Chronically-Infected Segmental defect in the Rat Femur Treated with BMP-2 and Local Antibiotic

10. Adjunctive Care for the Prevention of Acinetobacter-induced Osteomyelitis Using a Fast-Acting Local Delivery System

11. Modification of an Accepted Animal Model of Osteomyelitis to Simulate and Evaluate Treatment of War Extremity Wounds

12. Antibiotic Impregnated Bone Cement for the Treatment of Osteomyelitis and Severe Open Fractures: Expanded Options for Surgeons

13. Serum and Exudate Calcitonin Precursors as Predictors of Wound Infection and Dehiscence in Wartime Penetrating Injuries

14. A Protocol to Improve Outcomes of High energy, Contaminated Wounds

1. **Project Title:** Mechanisms of Heterotopic Ossification and Characterization of Preventive Therapeutics

PI Name: Maurizio Pacifici, PhD
Institution: Thomas Jefferson University, Philadelphia, PA
Department: Orthopaedic Surgery

Background: (*Verbatim from the Applicant)* Heterotopic ossification (HO) is triggered by severe trauma, extended immobilization, burns or major surgical interventions, conditions that affect a significant segment of our military particularly in the current time of war. HO pathogenesis is poorly understood and current treatments are ineffective. Formation of HO lesions closely resembles processes occurring during normal fetal skeletogenesis, including local production of skeletal-inducing factor(s), recruitment of mesenchymal progenitor cells, and differentiation of cartilage and bone.

Objective/Hypothesis: One of the mechanisms regulating fetal skeletogenesis is the retinoid signaling pathway and its nuclear transcription factor effectors (RARs and RXRs). Previous studies from this and other groups showed that experimental activation of retinoid signaling blocks fetal skeletogenesis. In Preliminary Studies using a HO mouse model, we now find that a synthetic retinoid agonist strongly inhibits skeletal cell differentiation and HO lesion formation. **Our hypothesis is that selective pharmacological activation of retinoid signaling inhibits activity of skeletogenic factors, blocks HO-associated progenitor cell differentiation and may thus represent a novel and specific treatment for HO.**

Specific Aims: In **Aim 1** we will test effectiveness of different retinoid agonists and routes/modes of administration on HO prevention. In **Aim 2** we will characterize the nature of HO lesion-producing cells and genes involved in their differentiation, and determine at which of these levels the retinoids act to block HO. We will pay particular attention to the ACVR1 gene recently found to cause an extreme form of HO. In **Aim 3** we will create a model of human HO by implanting bone marrow-derived skeletogenic cells in nude mice and will test whether the retinoid agonists can block human HO lesion formation. We will also compare cells obtained from different individuals for possible differences in retinoid sensitivity.

Study Design: The project makes use of cellular, biochemical and molecular approaches to decipher the mechanisms involved in HO and test the effectiveness of retinoid-based drug treatments to prevent or reverse HO lesions. It may also produce a screening test in which human cells from different individuals are tested for therapeutic responsiveness.

Relevance: HO remains a pervasive and debilitating condition. Its pathogenesis is poorly understood and current treatments are far from ideal. Pharmaceuticals taken orally or subcutaneously (such as those studied here) could provide novel, mechanistically-based, easily administered and early-intervention therapeutics in the hands of clinicians or other support personnel under combat and non-combat circumstances.

2. **Project Title:** Polymicrobial Chronic Infection Including Acinetobacter baumannii in a Plated Segmental Defect in the Rat Femur

PI Name: Dean T. Tsukayama, MD
Institution: Minneapolis Medical Research Foundation; Minneapolis, MN
Department: Medicine, Division of Infectious Disease and Internal Medicine

Background: (*Verbatim from the Applicant)* The majority of the combat casualties in Operations Iraqi Freedom and Enduring Freedom are a result of high-energy blast or high-velocity projectile mechanisms, and commonly present with a significant segmental bone defect, massive soft tissue disruption, and substantial contamination with bacteria. The goal of this study is to develop a model of a polymicrobial chronic infection in an internally-stabilized segmental defect in the rat femur. This model will then be used to study the effect of debridement and local antibiotic to treat the chronic infection.
Specific Aims: We will first perform an initial screening to determine a contaminating inoculum of *Acinetobacter baumannii* (Aim #1) and a time from contamination that would reliably produce an infection in an internally-stabilized segmental defect, yet not cause the animals to become septic, and not cause a significant amount of bony lysis that would seriously compromise defect fixation. In Aim #2, we will assess the effectiveness of treatment of this chronic infection by surgical debridement with and without local antibiotic therapy with Gentamicin. Aim #3 will repeat Aim #1 except that combinations of inocula of *A. baumannii* and *S. aureus* will be screened. Finally in Aim #4, treatment of this polymicrobial infection with debridement with and without local administration of Gentamicin will be assessed.
Objective/Hypothesis: We hypothesize that there is a combination of inoculum and time from contamination that will consistently result in an infection in all animals, will not cause the animals to become septic, will provide definable material for debridement, and will cause a detectable amount of bony lysis, but not an extensive amount that would dramatically reduce fixation stability. It is also hypothesized that local antibiotic therapy with surgical debridement will reduce the number of bacteria recovered from the defect, and will lead to less bony lysis and less reduction in fixation stability, compared with treatment with no antibiotic.
Study Design: Internally stabilized segmental defects will be contaminated with various inocula of *A. baumannii* alone (Aim #1, 90 rats), and in combination with *S. aureus* (Aim #3, 75 rats). An inoculum and time from contamination will be subjectively determined that will produce an infection, yet not cause a significant amount of bony lysis that would seriously compromise defect fixation. In Aims #2 and #4, segmental defects will be contaminated with inocula determined in Aims #1 and #3, respectively. The defects will be surgically debrided after a period of time determined in Aims #1 and #3, and will be treated with placement of a carrier with and without Gentamicin. At 2, 4 and 12 weeks after debridement, the progression of the infection and its treatment will be quantified by determining the number of bacteria recovered from the defect, torsional stiffness of the defect fixation, and radiographic evidence of bony lysis.
Relevance: This study will provide direct translational information to optimize the use of local antibiotics and commercially available bone graft materials or carriers to deliver these antibiotics, for improved treatment of infected segmental bone loss which frequently occurs in combat casualties.

3. **Project Title:** Novel Therapies for Acinetobacter Osteomyelitis

PI Name: Edward M. Schwarz, PhD
Institution: University of Rochester, Rochester, NY
Department: Orthopaedics

Background: (*Verbatim from the Applicant)* It has been well established from current casualty data of our military operations in the Middle East that the ratio of serious injuries to fatal casualties far exceeds that of previous conflicts. Among these serious injuries, war wound infection and osteomyelitis (OM) appear to be of greatest concern. Most alarming is the incidence of multidrug-resistant (MDR) Acinetobacter species. This comes as a major surprise since this pathogen has been reported in less than 2% of nosocomial infections within the United States, but has emerged in over 30% of admitted deployed soldiers. An additional problem is that while there are some effective antibiotics against Acinetobacter (i.e. Colistin and Imipenem), they are not available in bone void fillers that are primarily used to treat OM caused by Staphylococcus. To address this urgent need we propose a collaboration that will take advantage of the first quantitative animal model of implant associated osteomyelitis developed for Staphylococcus, and clinical isolates of MDR Acinetobacter obtained from our soldiers.
Objective/Hypothesis: The goals of this proposal are to evaluate the efficacy of Acinetobacter-specific antibiotics in a mouse model of OM and to understand the immune response to this unique pathogen during the establishment of infection. To achieve these goals we will test the hypotheses that: 1) prophylatic chemotherapy with Acinetobacter-specific antibiotics can prevent the establishment of Acinetobacter OM; 2) incorporation of Acinetobacter-specific antibiotics into polymethylmethacrylate bone void filler prevents OM in a contaminated wound; and 3) specific antibodies are raised against common immuno-dominant antigens during the establishment of Acinetobacter OM.
Specific Aims: (1) To develop quantitative outcome measures (BLI, micro-CT, RTQPCR and histomorphometry) to assess the in vivo bacterial load of Acinetobacter in our mouse model of implant-associated osteomyelitis, (2) To evaluate the efficacy of Acinetobacter-specific antibiotics (Colistin and Imipenem) as prophylatic chemotherapies and local therapy in bone void filler, and (3) to identify the immuno-dominant antigens of Acinetobacter.
Study Design: We have developed first quantitative animal model of OM whose outcome measures include: bioluminescent bacteria that can be analyzed in vivo using bioluminescent imaging (BLI), real time quantitative PCR (RTQ-PCR) of specific bacterial genes to quantify bacterial load in tissues, micro-CT to quantify osteolysis and histology of bacteria and osteoclasts. First we will adapt this model for Acinetobacter by transforming clinical isolates with the lux genes to obtain bioluminescent strains; and develop RTQ-PCR for Acinetobacter specific genes. Then we will use these quantitative outcome measures to assess the efficacy of systemic and local Acinetobacter-specific antibiotics. Finally, we will analyze the sera of infected mice to identify the common immuno-dominant antigens between various MDR strains of Acinetobacter isolated from our soldiers.
Relevance: The emergence of MDR Acinetobacter OM in our soldiers that have been deployed to Operation Iraqi Freedom and Operation Enduring Freedom (OIF/OEF) is alarming. Of additional concern is the fact that none of the clinically available antibiotic impregnated bone void fillers, which contain Gentamicin, Tobramycin or Vancomycin, are effective against Acinetobacter. Thus, development of Acinetobacter-specific antibiotic treatments to prevent the establishment of OM are needed for our soldiers in the theater of operations. Furthermore, towards the development of an effective vaccine, we need to understand the host immune response to this unique pathogen during the establishment of OM.

4. **Project Title:** Development of Military Field/Hospital Therapeutic Anti-Infective, Antibiofilm Acute Wound Care Gel Product

PI Name: Brett Baker, MSc, DC
Institution: Microbion Corporation, Bozeman, MT

Background: (*Verbatim from the Applicant)* Standard care for acute open wounds sustained in military personnel in Iraq and Afghanistan is vigorous irrigation and debridement, followed by packing of the wound with saline soaked gauze. Infections associated with open wounds sustained in military conflict are often difficult to treat with I.V. antibiotics alone, particularly as major wound pathogens are increasingly multiply antibiotic-resistant. Even when open wounds appear suitable for closure, infections frequently follow wound closure. There is a vital need for improved methods of treatment of acute, complex open wounds, to prevent infection, to prevent biofilm formation within the wound, and to promote rapid healing.

Objective/Hypothesis: The current standard of care for open wounds, vigorous irrigation and debridement procedures, do not (alone) promote the best chances of healing from acute wounds, particularly acute, complex military wounds and associated infections. We propose to test the hypothesis that a therapeutic, topical acute wound care gel containing bismuth-thiols (BTs) is capable of reducing the likelihood of infection following acute traumatic open wounds. The BTs are known to inhibit bacterial growth, prevent biofilm formation, and potentiate the effects of many important classes of antibiotics, including antibiotics known currently to have reasonable activity against *Acinetobacter baumannii*, a microorganism with particular relevance to military wounds.

Study Design: Preclinical tests, including efficacy, metabolism, range-finding studies (both in vitro and in vivo), and pharmacokinetics in one rodent model (mice) and one non-rodent model (goat) are intended to provide important, pragmatic information facilitating in vivo proof of concept and achievement of important regulatory requirements.

Relevance: The active component of the gel is a potent biofilm prevention agent, but importantly, it also facilitates key activities of antibiotics, such as overcoming antibiotic resistance. When combined at subinhibitory concentrations with multiple classes of antibiotics, the BTs are known to sensitize otherwise resistant bacteria, while also effectively preventing biofilm formation. In addition, the BTs reduce antibiotic microbicidal, inhibitory, and biofilm prevention concentrations. It is anticipated that this targeted topical product will become a vital therapeutic adjunct, potentiating current procedures and improving clinical outcomes for military and civilian patients suffering from contaminated open wounds.

5. **Project Title:** Prevention and Treatment of Heterotopic Ossification

PI Name: Alan R. Davis, PhD
Institution: Baylor College of Medicine, Houston, TX

Abstract: (*Verbatim from the Applicant)* Heterotopic ossification (HO), the formation of bone at a site where bone does not normally form, is a major problem in combat casualties in that it is painful, prevents proper fitting of prostheses, and the heterotopic bone must eventually be removed by additional surgery. We have developed a model of heterotopic ossification in mouse using BMP2 and have studied this model in detail to allow a choice of molecular/ cellular targets, which are likely to be unique to HO. Two therapies are described 1. inhibition of BMP-mediated bone formation by the use of noggin, since it has recently been shown that the rare genetic disease, FOP, where bone forms in muscle, is caused by mutations in the BMP receptor type I ACVR1. 2. delivery of leptin to burn the fat in local adipocytes, since we have shown that adipocytes burn oxygen in the local environment providing the hypoxia for the initial stages of HO including cartilage formation. In later phases, safety features added to ensure approvability, including use of regulated expression, as well as encapsulation of the producing cells in hydrogel so that they remain localized. All product development will be performed according to GLP guidelines.

6. **Project Title:** Improved Healing of Infected Segmental Bone Defects through Controlled Delivery of FGF-2, PDGF, and Tobramycin

PI Name: Scott A. Guelcher, PhD
Institution: Vanderbilt University; Nashville, TN
Department: Chemical Engineering

Background: (*Verbatim from the Applicant)* Restoration of bone form and function is achieved through the physiological and regenerative process of bone healing, which occurs when the fracture is reduced and stably fixed. Currently available bone grafting therapies have recognized limitations, particularly in the presence of infection. The aim of this grant application is to design and develop a therapy that will deliver growth factors and antibiotics to enhance bone defect healing and prevent/treat infections. The project deliverable will comprise a preclinical composite biomaterial to enhance healing of infected segmental bone defects.

Objective/hypothesis. The project hypothesis is that the poly(ester urethane)urea/microparticle-PDGF-FGF-2-tobramycin (PEUUR/MP-PDGF-FGF-2-T) therapy will enhance healing of infected segmental bone defects. **Technical aims**. The technical objectives comprise a systematic, focused process for designing and developing a therapy to enhance bone defect healing. *In vivo* bone healing studies will feed information back to materials design and development, thereby yielding an interactive set of aims directed toward therapy development. ***Technical Aim 1 (years 1)***. Design and develop a family of PEUUR/MP-T implants that promote healing in an established infection model. ***Technical Aim 2 (years 1-2).*** Design and develop a family of PEUUR/MP-BMP-2 implants that promote bone regeneration in an established rat femur critical size defect model. ***Technical Aim 3 (years 1-3)***. Design and develop a family of PEUUR/MP-FGF-2 and PEUUR/MP-PDGF implants that promote bone regeneration in an established rat femur critical size defect model. ***Technical Aim 4 (years 3-4)***. Design and develop a family of PEUUR/MP-PDGF-FGF-2 implants that promote bone regeneration in an established rat femur critical size defect model. ***Technical Aim 5 (years 5)***. Design and develop a PEUUR/MP-PDGF-FGF-2-T implant that enhances bone healing in an established infected wound model.

Study design. New composite delivery systems will be prepared using known chemical synthesis techniques. Standard materials characterization, molecular biology, and histology techniques will be used to assess outcomes.

Relevance. The project goal is to develop a biocompatible, biodegradable composite biomaterial that enhances healing of infected segmented bone defects. If successful, this would represent a significant advancement in the development of bone healing treatments.

7. **Project Title:** Adipose-derived Mesenchymal Stem Cells for Treatment of Large Bone Defects

PI Name: Barbara D. Boyan, PhD
Institution: Georgia Institute of Technology, Atlanta, GA
Department: Biomedical Engineering

Background: **(***Verbatim from the Applicant)* Trauma-induced injuries being sustained by our military men and women result in loss of multiple tissues, including associated vasculature and nerves. Current replacement technologies cannot address these kinds of injuries. Tissue engineering is still largely at the single cell stage and the tissues that are produced do not integrate well with surrounding tissues. Mesenchymal stem cells (MSCs) are an important tool but patients with large wounds may have reduced sources of stem cells and those stem cells that are present may be less robust as a consequence of trauma and medical treatment to suppress infection and inflammation.

Objective/Hypothesis: Our proposal is based on the hypothesis that effective repair of large defects requires the concerted processes of bone modeling and remodeling to create a functional marrow cavity, vascularization, and innervation and the best way to achieve this is by using autologous stem cells. Our objective is to develop technology that will enable us to use adipose-derived MSCs to treat critical size segmental defects.

Specific Aims: Adipose-derived MSCs are attractive because of their relative abundance but there are still many issues that need to be resolved to ensure that these cells can be used effectively. Our specific aims are to: (1) develop methods for enriching the population of MSCs in adipose-derived cell preparations from rats; (2) determine if MSC-enriched adipose cells can be used to effectively treat large segmental defects using a rat segmental defect model developed in our group; and (3) optimize this technology for use in male and female animals.

Study Design: Adipose tissues from male and female normal Sprague Dawley rats and obese Zucker rats will be used as a source of cells. MSCs will be enriched by selective removal of adipocytes and the effectiveness of this strategy will be determined using in vitro and in vivo assays. To test the ability of enriched MSCs to repair a critical size defect, cells will be loaded onto polymer composite scaffolds and implanted in a rat segmental defect (male in male; male in female; female in male; female in female). Healing will be assessed by microCT and by histology, histomorphometry, immunohistochemistry, and biomechanical testing.

Relevance: This research will provide important new technology for treatment of bone injuries due to trauma. Most individuals have an adequate supply of fat tissue that can be used as a source of cells, but MSCs are in low abundance. Culture expansion dilutes MSCs with more committed cells. In contrast, our approach is to enrich the MSCs in the population by selective removal of more differentiated adipocytes.

8. **Project Title:** Cellular Therapy to Obtain Rapid Endochondral Bone Formation

PI Name: Elizabeth Olmsted-Davis, PhD
Institution: Baylor College of Medicine, Houston, TX

Abstract: (*Verbatim from the Applicant)* The goal of this study is to provide a safe effective system for inducing bone formation for fracture healing. This set of proposed experiments will provide significant knowledge to the field of bone tissue engineering. Proposed studies will provide essential biological information and involves the development of a novel biomaterial that can safely house the cells expressing the bone inductive factor to produce the new bone at which time the material is then selectively eliminated. Ultimately this system has significant applicability. Often bone graft must be surgically removed from the pelvis, to implant into the site of difficult fractures for proper healing. This additional surgery often results in significant pain, and long term healing. Further, this system would be applicable to orthopedic trauma situations that previously resulted in amputation. We propose in these studies to complete the development of this bone induction system and test it in a preclinical animal model. Validation of our hypothesis will provide a safe and efficacious material for the production of bone leading to reliable fracture healing, circumventing the need for bone grafts, or for direct administration of cells, viruses, or other materials that could lead to significant adverse reactions.

9. **Project Title:** New Bone Formation in a Chronically-Infected Segmental defect in the Rat Femur Treated with BMP-2 and Local Antibiotic

PI Name: David W. Polly, Jr., MD

Institution: Minneapolis Medical Research Foundation, Minneapolis, MN

Department: Orthopaedic Biomechanics Laboratory

Background: (*Verbatim from the Applicant)* The majority of the combat casualties that occur in Operations Iraqi Freedom and Enduring Freedom are a result of high-energy blast or high-velocity projectile mechanisms, and commonly present with a significant segmental bone defect, massive soft tissue disruption and loss, and substantial contamination with bacteria. The goal of this research is to improve the treatment of infected segmental bone loss by leveraging an existing rat model of a chronically-infected segmental defect and currently available off-the-shelf biologics and antibiotics.

Specific Aim: Demonstrate whether human recombinant bone morphogenetic protein-2 (rhBMP-2) and the antibiotic Cefazolin delivered locally from a composite Absorbable Collagen Sponge (ACS)/MasterGraft Matrix carrier can lead to new bone formation in an internally-stabilized rat femoral segmental defect with a *Staphylococcus aureus* chronic infection.

Objective/Hypothesis: We hypothesize that (i) chronically-infected defects treated with debridement and rhBMP-2 will form significantly more and stronger new bone than debrided defects without rhBMP-2, and (ii) defects treated with debridement, rhBMP-2 and local administration of a high dose of antibiotic will form significantly more and stronger new bone than debrided defects treated with rhBMP-2 alone.

Study Design: Our established chronic infection model will be used. Specifically, a 6 mm segmental defect of critical size will be surgically created and stabilized with a polyacetyl plate and 6 Kirschner wires in the left femur in each of 360 Sprague-Dawley rats. All defects will be contaminated with 10_4 colony-forming units of *S. aureus*. The animals will be allowed to recover for 2 weeks during which time the initial contamination has been shown to progress to a chronic infection. All defects will then be thoroughly debrided under sterile conditions, and treated with 0, 20 or 200 µg of rhBMP-2 in a composite ACS/Matrix carrier, with and without a 100 mg dose of Cefazolin. Animals will be euthanized at 2, 4, 8, or 12 weeks after debridement. The amount and strength of the newly mineralized callus will be assessed with micro-computed tomography, torsional failure testing, and undecalcified histology.

Relevance: This study will provide direct translational information to optimize the use of commercially available bone graft materials, BMPs and antibiotics. These will be stand-alone results for improved treatment of infected segmental bone loss which frequently occurs in combat casualties.

10. **Project Title:** Adjunctive Care for the Prevention of Acinetobacter-induced Osteomyelitis Using a Fast-Acting Local Delivery System

PI Name: Warren O. Haggard, PhD
Institution: The University of Memphis, Memphis, TN
Department: Biomedical Engineering

Abstract: (*Verbatim from the Applicant)* Military operations in Iraq and Afghanistan have caused many open fractures resulting from high energy trauma. With these injuries, wound infections are common, resulting from bacterial contamination, including contamination with *Acinetobacter*, a bacterial species commonly found in water and soil. This exposure has led to an increase in *Acinetobacte*r-induced osteomyelitis and nosocomial infections in military hospitals abroad and in the United States, as hospitalized patients are at increased risk for infection. This bacteria can affect multiple organ systems, and there are several strains of multidrug-resistant (MDR) Acinetobacter species, including *Acinetobacter baumannii*. Current treatment to lessen or prevent *Acinetobacter*-induced osteomyelitis utilizes surgical debridement, irrigation or lavage treatments, and systemic antimicrobial therapy with imipenem and high dose (15-20 mg/kg daily) amikacin. This combination therapy decreases the risk of developing more highly resistant organisms, which can contribute to the nosocomial infection risk. An adjunctive therapy to current treatment will be studied in this proposal. This adjunctive therapy will utilize an early local delivery system to deliver appropriate antibiotics at the trauma site after initial surgical debridement and irrigation at a combat support hospital. The delivery system will provide high local levels of amikacin, but low systemic levels. This delivery system will be composed of calcium sulfate, engineered to dissolve rapidly after application to the wound, delivering antibiotics to the trauma area in a simple and efficient manner. Calcium sulfate is a proven biomaterial and has many applications in the bone grafting and drug delivery fields.

11. **Project Title:** Modification of an Accepted Animal Model of Osteomyelitis to Simulate and Evaluate Treatment of War Extremity Wounds

PI Name: Jason H. Calhoun, MD
Institution: University of Missouri-Columbia, Columbia, MO
Department: Orthopaedic Surgery

Background: (*Verbatim from the Applicant)* Strains of bacteria resistant to tested antibiotic therapies are of increasing concern in casualties from Iraq and Afghanistan. The choice, timing, and duration of antibiotic are crucial to ensuring effectiveness and reducing the growth of resistant strains.

Objective/Hypothesis: We hypothesize that the rabbit model of osteomyelitis can be used to demonstrate the effectiveness of selected antibiotics as prophylaxis against these infectious agents in a simulated blast wound.

Specific Aims: 1.) To collect data and prepare a merged database of pathogens observed in infected wounds at military medical centers, leading to selection of specific bacteria to be subject to animal testing. 2.) Modification of the rabbit osteomyelitis model to better simulate infections in blast wounds. 3.) Execution of the rabbit osteomyelitis study of efficacy of selected antibiotics for infections in simulated blast wounds in both preventive and treatment approaches.

Study Design: We will conduct a series of experiments using the established rabbit model for the study of osteomyelitis, first to determine the pathogenecity of 1.) *Acinetobacter baumannii*, 2.) *Pseudomonas aeruginosa* and 3.) *Klebsiella pneumoniae*; second to add methicillin-resistant *Staphylococcus aureus*; and third to test combinations of multiple infections of all four pathogens. The final experiments will modify the rabbit model to simulate blast wounds and study the four infectious agents above for both treatment and prophylaxis.

Relevance: There is a crucial need for more information regarding the types of infections seen in personnel from Iraq and Afghanistan. Modification of the rabbit model will provide a new pathway for researchers to study this problem, and the study will in the shorter run offer practical insights to guide recommendations for the use of antibiotic prophylaxis in battlefield wounds.

12. **Project Title:** Antibiotic Impregnated Bone Cement for the Treatment of Osteomyelitis and Severe Open Fractures: Expanded Options for Surgeons

PI Name: LCDR Michael Mazurek, MD
Institution: Naval Medical Center San Diego, San Diego, CA
Department: Orthopaedics

Background: (*Verbatim from the Applicant)* Local antibiotic delivery via bone cement is an important adjunct in the treatment of osteomyelitis and severe open fractures. The body of literature addressing antibiotic impregnated bone cement largely deals with options for pathogens typically encountered in endoprosthetic infections. In today's era of emerging multiple drug resistant bacteria and atypical infections encountered in severe open fractures and contaminated war wounds, there is a need for further guidance on available antibiotic bone cement options for the orthopaedic trauma surgeon.

Objective/Hypothesis: The proposal will determine stability, elution profiles and pharmacodynamic properties in bone cement of available solid form antibiotic and antifungal medications in an effort to establish a reference of local antibiotic delivery options for treating osteomyelitis and contaminated open fractures. Emphasis will be directed towards establishing viable antibiotic combinations in bone cement for the treatment of multiple drug resistant organisms encountered in severe open fractures and contaminated war wounds.

Study Design: This proposal is an *in vitro* study that will examine the stability, elution profiles, and pharmacodynamic properties of solid form antibiotic and antifungal medications in polymethylmethacrylate cement beads immersed in a phosphate buffered solution. Liquid-chromatography-tandem mass spectrometry will be used to analyze and quantify antibiotics in the bathing medium at regular intervals. Pharmocodynamic analysis will be performed using quantitative dilution methods as described by the Clinical and Laboratory Standards Institute.

Relevance: The proposal will provide a comprehensive reference of antibiotic and antifungal bone cement options for treating osteomyelitis and severe open fractures and will establish viable antibiotic combinations for local treatment of multiple drug resistant organisms commonly seen in contaminated war wounds and severe open fractures. The data obtained from this proposal can immediately be used to guide therapy with antibiotic impregnated bone cement. Furthermore, data from this project can be used for future *in vivo* research and investigations exploring the manufacturing and storage of specific antibiotic bead preparations for possible use in the war theatre.

13. **Project Title:** Serum and Exudate Calcitonin Precursors as Predictors of Wound Infection and Dehiscence in Wartime Penetrating Injuries

PI Name: Jonathan A. Forsberg, MD
Institution: National Naval Medical Center, Bethesda, MD
Department: Orthopaedic Surgery

Abstract: (*Verbatim from the Applicant)* The treatment of wartime penetrating injuries accounts for a significant amount of time and resources directed toward either limb salvage or preservation. Serial debridements are carried out to remove devitalized tissue and eradicate infection. Local antibiotic delivery, high pressure irrigation and wound evacuation dressings have advanced the treatment of these injuries, but the decision to primarily close or perform flap coverage of a wound remains subjective. Considerable intra-observer variability exists and despite meticulous debridements and antibiotic therapy, some clean appearing wounds go on to dehiscence and infection. Conversely, because of this uncertainty, benign appearing wounds may undergo unnecessary surgical debridements, exposing patients to additional anesthesia risks and surgical morbidity. A serum or exudate marker that correlates with wound dehiscence and infection could prevent life and limb-threatening complications caused by premature wound closure and eliminate the morbidity associated with unnecessary debridement procedures. Current serum and exudate markers are poor predictors of local infection recrudescence and wound dehiscence, especially for individuals with multiple injuries. An exudate marker that could guide surgical decision making for a specific extremity would be particularly valuable in the treatment of battlefield extremity trauma.

Recently, calcitonin precursors (CTprs) have emerged as ultraspecific indicators of infection, response to therapy, and severity of infection in a variety of conditions including multiple trauma, sepsis and burns. Current research in this area focuses on serum analysis in severe, generalized infections and sepsis. Recent literature suggests that CTprs, including procalcitonin (ProCT) and other cytokines may be elevated in the serum of patients with localized infections, commonly found in wartime extremity wounds. Exudative analysis of ProCT has not been described. Until recently, assays were not sensitive enough to detect ProCT in the serum or exudate in patients with extremity wounds and/or localized infections. With newer assay techniques, serum and exudative analysis of ProCT/cytokine levels may provide an objective means to assess the degree of bacterial inflammation and confirm eradication of a local wound infection. This project will demonstrate that ProCT and other cytokines are detectable in wound exudate. It will also determine the sensitivity, specificity, positive and negative predictive values of serum and exudate ProCT/cytokines with respect to wound dehiscence and infection. Finally, it will compare the efficacy of serum or exudate ProCT/cytokine levels to established serum or exudate markers for infection in predicting the risk of wound infection and dehiscence.

14. **Project Title:** A Protocol to Improve Outcomes of High energy, Contaminated Wounds

PI Name: Lawrence X. Webb, MD
Institution: Wake Forest University Health Sciences, Winston-Salem NC
Department: Orthopaedic Surgery

Background: (*Verbatim from the Applicant)* In high energy contaminated wounds, microcontaminants and bacteria are retained within the wound setting the stage for further tissue necrosis and infection. Current traumatic wound care removes dead tissue and foreign particulate matter from the wound (debridement) leaving viable but tenuous wound tissue with a variable level of microcontaminants and bacteria.

Objective/Hypothesis: The proposed clinical trial will evaluate the removal of contaminated/dead tissue using vacuum jet debridement and active resuscitation of the wound's zone of stasis by application of topical negative pressure at the wound site. We hypothesize that combining these two methods will result in a decreased bacterial load 48 hours after debridement, decreased incidence of wound infections, decreased requirement for tissue flaps, and decreased saline usage during debridement.

Study Design: Two different wound management techniques will be evaluated. One surgeon will use a combination of vacuum jet debridement followed by the application of a topical, negative pressure dressing. The other surgeon will use conventional debridement techniques, pulsatile lavage, and traditional wet-to-dry dressing changes.

Relevance: The majority of traumatic injuries sustained by soldiers involved in Operation Iraqi Freedom and Operation Enduring Freedom are orthopaedic-related high energy wounds contaminated with microcontaminants and bacteria. The unique combined use of vacuum-based debridement and a negative pressure dressing will improve wound management and subsequent healing of these devastating injuries.